Chapter 6
Enzymatic Mismatch Cleavage and Agarose Gel Evaluation of Samples

Abstract Denaturation and annealing of PCR products allows DNA strands with small sequence differences to hybridize together. The result is heteroduplexed molecules that are single stranded in polymorphic sequence locations, but double stranded elsewhere. These molecules are the substrates for cleavage by single-strand-specific nucleases such as CEL I, crude Celery Juice Extract (CJE) containing CEL I, and other plant extracts containing single-strand-specific nucleases [Till et al. (Nucleic Acids Res, 32:2632–2641, 2004)]. Enzymatic cleavage initiates on a single strand and can result in double strand breaks. The products of cleavage can therefore be observed using native gel electrophoresis.

6.1 Materials

Consumables and equipment for enzymatic mismatch cleavage are listed in Table 6.1.

6.2 Methods

1. Prepare the following enzyme master mix on ice (calculated for five samples):
   81.5 µl water
   15 µl 10× CEL I buffer
   3.5 µl CJE nuclease
2. Label four new PCR tubes with the sample name.
3. Combine 20 µl of PCR product with 20 µl of enzyme master mix. Pipette the mixture up and down to mix or vortex briefly followed by pulse centrifugation.
4. Incubate at 45 °C for 15 min in a thermal cycler.
5. Place the reactions on ice, stop the reaction by adding 10 µl of 0.25 M EDTA per sample, and mix well by vortexing and centrifuge briefly (NOTE: Samples can be stored frozen for months before analysis).

B.J. Till et al., *Low-Cost Methods for Molecular Characterization of Mutant Plants*,
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Table 6.1 Chemicals, enzymes, and equipment for enzymatic mismatch cleavage

Material description	Examples of suppliers and specifications
10× CELI buffer	5 ml 1 M $MgSO_4$, 100 µl 10 % Triton X-100, 5 ml 1 M Hepes (pH 7.4), 5 µl 20 mg/ml bovine serum albumin, 2.5 ml 2 M KCl, 37.5 ml water
Crude Celery Juice Extract (CJE)	See Till et al. (2004) for the preparation of enzyme and defining unit activity. Chap. 7 provides a protocol for the preparation of single-strand-specific nucleases from weedy plants
1 kb DNA ladder	Any general laboratory supplier
0.25 M EDTA	Prepared from ethylenediaminetetraacetic acid (EDTA) stock from any general laboratory supplier
H_2O	Distilled or deionized and autoclaved
1.5 ml, 2.0 ml tubes	Any general laboratory supplier
Thermocycler	e.g., Biorad C1000 Thermal cycler
Microcentrifuge	Eppendorf Centrifuge 5415D
Agarose gel equipment	Horizontal electrophoresis from any general laboratory supplier

6. Analyze the samples by electrophoresis using a 1.5 % agarose gel. See Chap. 8 for example data.

Reference

Till BJ, Burtner C, Comai L, Henikoff S (2004) Mismatch cleavage by single-strand specific nucleases. Nucleic Acids Res 32:2632–2641